After ten hours in April, we have fairly large increases all the way through October. There are approximately two logs, or 75 fold increases is what we have seen during the ten hours.

With the assistance of John Bowers (phonetic) and some fancy math. He's taken the data from April through October and produced a gauntlet curve. What this shows is that the average levels were about 100 per gram and very quickly they reached the exponential phase, and by about 20 to 24 hours they have reached their maximum growth.

The next slide summarizes the data. We observed a lag time of about one hour, after which they doubled slightly less than every hour-and-a-half. By the end of the 24 hour period they had increased about 1500 fold or slightly more than three logs.

One of the limitations, and it seems like everything we do has limitations, is that when you deal with natural populations, as we were in the other study, they're comprised primarily of non-pathogenic strains. Of course the question is, do the virulent or pathogenic strains behave similarly to the total populations, are the total populations an indicator of this? Both at Chuck's lab and our lab there are studies, preliminary data and

ongoing studies, that are addressing this question.

This is some study. Because the pathogenic strains occur so infrequently in oysters what was done we achieved incurred levels by placing the oyster in aquaria and adding the pathogenic strain to the water and allowing the oysters to accumulate naturally through the filter-feeding process.

At the beginning of the study the levels of 10,000 were what was seen in the west coast oysters. Then they were stored at several different temperatures. What we see at 95 Fahrenheit, which is about 35 C, is that within 24 hours they had increased three logs and reached maximum levels, and did not increase beyond that.

With the lower temperatures of 16 70 we see a little bit longer lag times and then slower growth rates.

This is some data that's being generated at our laboratory. We're doing the same thing except we're using not a west coast strain, but an 03:K6 strain, and we're storing at 26 degrees. There have been three trials with various dosing levels and what we've seen in all these is that you have about a three log increase by 24 hours. So I think that these data suggest that the pathogenic strains will grow in oysters and they have same maximum growth. We'll probably need to look at more sampling

points early on to establish lag and doubling times, and also to look at some other temperatures that are more representative of the climates in various areas of the country.

Refrigeration. We have been talking about temperatures that allow vibrio parahaemolyticus to grow and we know that if you get cool temperatures you can stop their growth. With vibrio vulnificus we know that you can achieve slight reductions with refrigeration. We are also currently investigating this, both on the west coast and on the Gulf Coast.

This the same sort of data with incurred levels of the pathogen. Here they were stored at 40 and 50 degrees. What we see is a period of several days where there is very little change in the numbers, and afterwards there are slight reductions through two weeks.

We've worked with natural populations on the Gulf Coast as an extension of Jan Guch's work, that I was describing just a few minutes ago. After holding the oysters at 26 degrees for 24 hours we then transferred them to three degrees for a couple of weeks. What we noticed was about a seven-fold reduction, a little less than one log reduction. Repeating this 12 times during each month of the year.

So, you can achieve some small reductions in vibrio parahaemolyticus by refrigeration.

The final segment to talk about is mitigation. Much of what I'm going to deal with here today has originally been proposed and implemented with vibrio vulnificus, which has a similar ecology as vibrio parahaemolyticus. There is a time/temperature matrix for refrigerating oysters after they're harvested. The depuration and relaying is something that's been done with fecally-associated pathogens, and the post-harvest processing include technologies that I'll describe in some of the later slides.

The time/temperature matrix is described in much more detail in the NSSP model ordinance. But, to summarize, states that have been implicated in multi vibrio vulnificus cases are required to have their oysters under refrigeration within ten to fourteen hours, depending on the water temperature, and this control is from April through October.

During the remaining part of the year they must have them under temperature control of 45 degrees within 36 hours.

The requirements are less stringent for other states, depending on the season they must be refrigerated

within 20 hours or 36 hours.

There's very little data on depuration of vibrio parahaemolyticus. The only study, and actually Chuck found this, was an ASM abstract in 1981, and we're not sure whether it was published or not. It was with hard-shell clams and it showed reductions of vibrio parahaemolyticus of approximately one log in three days. For those of you who are not familiar with depuration, this a process of usually taking oysters from a restricted area, placing them in the laboratory in controlled aquaria with either free-flowing or purified recirculating water. This is generally done for two days before they can be marketed.

What we know about vibrio vulnificus is that depuration doesn't work very well. This is because the bacterium multiplies in the oyster tissues. In fact, vibrio vulnificus, the oyster was shown to release one-million cells per day of vibrio vulnificus. And in fact, a lot of attempts to depurate this organism has actually resulted in increased numbers.

In a study we did in our lab a few years ago we took oysters from an approved area, this is similar to relay, which is normally moving them from a restricted to an approved area for two weeks. We took them offshore and

suspended them on a gas-rig in the Gulf of Mexico and were able to achieve reductions of less than ten per gram vibrio vulnificus, probably due to vibrio vulnificus' dislike of high-salinity water.

A similar approach may not be as effective for parahaemolyticus, as it tolerates higher salinities than vibrio vulnificus.

There's been a number of processing technologies that have been proposed and some have been -- are actually in use. These include a mild heat treatment, freezing followed by storage, and irradiation and hydrostatic pressure have also been proposed. These have been aimed primarily at reducing levels of vibrio vulnificus to less than three per gram, MPN that is. This is the NSSP definition of non-detectable. Plants that are doing this must have a HASSA (phonetic) plan, and if they are able to achieve this then they can label their containers as processed to reduce vibrio vulnificus to non-detectable levels, or they may be able to take the warning for vibrio vulnificus off of their containers.

Work that was published several years ago by one of our committee members and my boss, Dave and Angela here, show that you could reduce natural vibrio vulnificus populations by six logs simply by heating shucked oysters

for 50 degrees C for five minutes.

Unpublished work in our lab shows that vibrio parahaemolyticus has a similar heat sensitivity, not quite as sensitive as vulnificus, but nearly as sensitive. The company Ameri Pure has in fact patented a process using shellstock which reduces vibrio vulnificus to less than three per gram.

A second process that's being used by some industry is freezing. The same study that was done with the mild heat treatment showed that you could reduce vibrio vulnificus level by four to five logs by freezing and storing them for three to four weeks.

In a different study using shrimp homogenate vibrio vulnificus and vibrio parahaemolyticus were shown to have a similar survival during freezing.

One processor has recently applied to FDA for an approval of labeling and they have also made an additional claim that they can reduce vibrio parahaemolyticus to non-detectable levels. The agency is currently reviewing this to see if the data supports these claims.

One caveat to these post-harvest processing techniques is the ability of the organisms to adapt. In two recently published studies this has actually been seen. The first is with a vibrio vulnificus. When cells

were grown in a culture media and exposed to 15 degrees Centigrade briefly it increased their survival following up chilling or freezing, compared to cells that were not adapted at 15 degrees.

Another study that was done on vibrio parahaemolyticus the cells were exposed to a pH of six, which is not that much different than the pH of an oyster, and it increased their acid tolerance. It may increase their ability to survive the gastric barrier, but it also cross-protected them against low salinity and thermo inactivation. These probably need to be studied more carefully so that procedures that are intended to reduce vibrios to non-detectable levels can be optimized.

The main conclusion that I have is that market levels are higher than harvest levels. I think this has been shown by both the FDA data and the Florida data. The laboratory studies show the vibrio parahaemolyticus both natural populations and incurred pathogenic strains can multiply usually about three logs if they are not refrigerated.

The densities do decline slowly during a refrigerated storage, and large reductions in densities can be achieved by the mild heat treatment or the freezing procedures.

DR. MICHAEL JAHNCKE: Thank you. If there are any questions from the subcommittee members, remember to identify yourselves, but if there are any questions for Dr. DePaola. Yes, Dane.

MR. DANE BERNARD: Thank you. Dane Bernard. Andy, nice presentation. Thanks. You mentioned vibrio in the tissues of the oyster. Can you elaborate a little further about what tissues? We're obviously talking about outside the digestive tract. And, can you comment on whether that occurs prior to harvest, or is this an after-harvest, post-harvest phenomenon that we get location in tissues outside the digestive tract? Thanks.

DR. ANDY DEPAOLA: The work has been done with vibrio vulnificus and not with vibrio parahaemolyticus. Both the previous one and the one we completed a few years ago showed that the fluids, the hemolith and manna fluid contained lower levels of vibrio parahaemolyticus than the abductor muscle, the manna tissue, and the digestive organs were usually ten to a hundred-fold higher in vibrio vulnificus numbers, and this was at harvest.

DR. MICHAEL JAHNCKE: Dr. Buchanan?

DR. ROBERT BUCHANAN: Andy, you had some data on the growth response of vibrio at 26 degrees celsius. Is there available in the literature a mathematical model for

the effect of temperature at several temperatures that you can rely on?

DR. ANDY DEPAOLA: I'm sure there's not for oysters. There may be such models for tryptic soy broth or something along those natures, but that's certainly one of the research needs is to look at the effect of different temperatures. I think we've established 26, the growth patterns there fairly well. The last time I checked the water in Mobile Bay, which was Monday, it was 26 degrees exactly, and those temperatures generally are the kinds of temperatures we see on the Gulf May through October. Obviously the climates are different in the higher latitudes.

DR. ROBERT BUCHANAN: If there is not a model available for vibrio parahaemolyticus, is there a model for a surrogate organism that you could use in its place?

DR. ANDY DEPAOLA: Not really. I think the studies are easy enough, particularly now with the DNA probe method --

DR. ROBERT BUCHANAN: (interrupting) Again, we're not talking about future work, we're talking about what you need for July 6.

DR. ANDY DEPAOLA: No, we don't have any models that would be a good surrogate model.

DR. ROBERT BUCHANAN: Okay. Do you have any information available -- you've indicated that there's increased levels as a result of what appears to be between -- handling between harvest and marketing. Do you have any estimates on the percentage of thermal abuse that you could consider as a result of the distribution system? Or, is everything under 100 percent refrigeration?

DR. ANDY DEPAOLA: As we analyzed the retail data some was at restaurants and some was at wholesale. We can look at those differences and they may or not be available before July. We do know that the oysters are stored aboard vessels, and that's where we suspect most of the growth occurs. Now, the question is whether you cool them down and when you rewarm them do you have longer lag periods associated with these because of their stress from being chilled. We don't have good information on that.

DR. ROBERT BUCHANAN: So you don't have good information on the adequacy of the cold chain from harvest up through consumption.

DR. ANDY DEPAOLA: Just circumstantial.

DR. ROBERT BUCHANAN: Okay. Just a point of clarification for myself on the refrigeration requirements, is that refrigeration requirement per oyster or is there some volume of oysters that have to be, or is

that ambient temperature? For example, the time it takes to chill down an oyster is a lot different than it would be to chill down a big rack of oysters.

DR. ANDY DEPAOLA: I think that's a very astute observation. The requirement is that the oyster be placed under mechanical refrigeration. It does not refer to the internal temperature of the oyster.

In our studies, one of the reasons I think we didn't have as much as increase during the winter months is that sometimes we were taking these oysters out of water at ten degrees Centigrade, putting them in a 26 degree air incubator. We'd put a probe inside and it took six hours, with about fifty oysters, to go up to 26 degrees. Then they continued to grow after we put them in the refrigeration, I think, because it took them six more hours to go from 26 to 3. And in the industry sometimes you're talking about sacks that are stacked on top of each other almost as high as this room.

DR. ROBERT BUCHANAN: So do you have any estimates on what would be the rate of chilling that you can anticipate?

DR. ANDY DEPAOLA: I think that's going to vary quite a bit from one system to the next.

DR. ROBERT BUCHANAN: Okay.

DR. MICHAEL JAHNCKE: Other questions from subcommittee? Mel?

MR. MEL EKLUND: This is Mel Eklund. I have a question that's kind of indirectly related to risk assessment. What diseases does vibrio parahaemolyticus present for the oyster itself? Could this be -- we have the TDH or the Kanagawa phenomenon, could this be an advantage that the organism has in infecting and causing a disease in the oyster, which then later becomes a problem for us as humans?

DR. ANDY DEPAOLA: Well, I don't know if the organism does effect the oyster. It must have a very infectious dose, as we see very high levels of this organism circulating through the circulator system.

MR. MEL EKLUND: As I remember, in Seattle, after the 1997 outbreak, we had a meeting. I don't know if Chuck Kaysner is still here. I think Ken Shu (phonetic) had mentioned oysters becoming diseased with the vibrio parahaemolyticus.

DR. ANDY DEPAOLA: I don't know of any problems in aquaculture where vibrio parahaemolyticus has been associated with oyster disease.

DR. MICHAEL JAHNCKE: Dane, you have a question?

MR. DANE BERNARD: Yes, thanks. Dane Bernard.

Follow-up on the temperature discussion, just to clarify in my own mind. We have oysters that are coming out of water that can be 26 C. We have a relatively low population. However, once we store those oysters, or once we expose them to ambient temperature after harvest we have a lag period of about 1.1 hour, as I remember the slide, and then a generation time of less than two hours for getting much higher counts. What happens? There has to be a change in the physiology of the oyster that allows the population to increase unchecked. What's going on, Andy?

DR. ANDY DEPAOLA: Well, there's probably two contributions to the vibrio parahaemolyticus and other vibrios that we see in molluscan shellfish. Those that they bring in from the outside water through filter feeding, and those that are growing within its tissues or digestive system. As I said, the vibrio vulnificus, there were studies that showed that each oyster produced one-million cells per day. When you take the oyster out of the water, you're taking it out of equilibrium where it's discharging cells and bringing lower concentrations in and as the bacteria multiply there's no where for them to go. That's sort of my theory. I don't have the data.

DR. MICHAEL JAHNCKE: Other questions? Bob?

DR. ROBERT BUCHANAN: Again, just to learn a little bit about the state of knowledge in terms of potential intervention strategies. Are there available all the needed formulas for calculating thermo-resistance? Do you have D values and Z values and those kinds of things available to you? Or, is this going to be more on a, here's what's out there, we sort of have data available on the efficacy of this process?

DR. ANDY DEPAOLA: Dave Cook from our laboratory has been working on that for the last year or so. I don't know how close he is to publication, but he is doing thermo-death times for various strains of vibrio parahaemolyticus, using the 50 degree C. Right now we're not doing that much with the frozen -- with the low temperatures, but we are for the mild heat treatments.

DR. ROBERT BUCHANAN: Okay, thank you.

DR. MICHAEL JAHNCKE: Other questions? Yes, Bill?

MR. WILLIAM SVEUM: Bill Sveum. I have several questions about your last conclusion. How do consumers find those oysters after those types of treatments? Do they look at in the same perception as a fresh oyster, the mild or the freezing, the mild heat treatment?

DR. ANDY DEPAOLA: I don't know that I can speak

for all consumers. I prefer the raw ones myself. The companies obviously claim that you can't tell the difference when there are taste panels.

DR. MICHAEL JAHNCKE: Other questions from subcommittee members? Angela?

MS. ANGELA RUPLE: I was just going to sort of respond to that question in that when we did the initial work with the shucked oysters we did several taste panels. The panel couldn't really tell the difference between the heated oysters. Initially there were some differences in salinity, but you can overcome that just by adding some additional salt. I think there are similar studies that have been done by some of the companies that are doing these with taste panels.

DR. MICHAEL JAHNCKE: Bob Buchanan?

DR. ROBERT BUCHANAN: Bob Buchanan, FDA. Andy, a question. Your presentation focused on the left-hand side of the original flow chart. Is the working assumption here that the shucked oysters are not a problem and will not be included in the risk assessment?

DR. ANDY DEPAOLA: I think the epidemiology, and Marianne Ross will maybe touch on that, but the recent outbreaks, I think, have been -- the shellstock has been most frequently implicated. There are certainly cases

that have occurred as a result of shucked oysters. There are maybe several factors going in there that they're usually cooked and the fact that they have been stored on ice, which may reduce the numbers more than the 45 to 50 degrees that the shellstock are stored in, and then the point Chuck brought up earlier, that the pH is lower. But, most of the problems as far as I'm aware of, are them having been associated with shellstock.

DR. ROBERT BUCHANAN: Okay, so the assumption is then that you're not going to have to worry about that part of the industry.

DR. ANDY DEPAOLA: Worry less about it.

DR. ROBERT BUCHANAN: Consider it in your risk assessment.

DR. ANDY DEPAOLA: Yeah, I think the risk assessment would spend its efforts mostly focusing on shellstock.

DR. ROBERT BUCHANAN: Okay.

DR. MICHAEL JAHNCKE: Other questions? If not, thank you, Dr. DePaola.

The next part of the meeting I'd like to invite all the NAC members in the audience to come up to the tables and have a general committee discussion on the presentations. I'm seeing no rapid movement. I would

like to open this up to the NAC members for general discussion and comments and questions on the presentations. Dr. Potter?

DR. MORRIS POTTER: I guess I'm somewhat concerned about some of the work we've heard on temperature changes and the resulting declines in vibrio numbers. I wonder how much of the decline is laboratory artifact from stressed cells that aren't growing in the medium that's being used or in the test systems that are being used, when in fact those numbers -- we may not be getting the level of reduction from the treatment that we think, but rather just a reduction in the numbers we can grow up in our laboratory systems. Any sense from the subcommittee or other members of the National Advisory Committee on that?

DR. ROBERT BUCHANAN: I can't speak in terms of specifics related to vibrio species. Though similar work I am experienced with, with aeromonas, indicates that these organisms are very sensitive to injury. Thermo acid and even salt. I would have to go back and look at the individual papers, but if they went directly into any kind of selective enrichment I would assume that there would be a fairly large artifact associated with the assay methods. So any published data looking at thermo-resistance, et

cetera, you would have to be really careful that they did take into account that phenomenon, or it would greatly exaggerate the effectiveness of the system.

DR. MICHAEL JAHNCKE: Dane?

MR. DANE BERNARD: Just an overall impression that leads to a question. Dane Bernard, by the way, for the record. The data presented seems to indicate that those who eat raw molluscan shellfish do come across vibrios fairly routinely, I guess. Has there been a speculation, I know it will be part of the output of the risk assessment, but how often one would consume vibrio parahaemolyticus and/or vibrio vulnificus, and how often that results in human illness? Any speculation so far?

DR. MORRIS POTTER: Dr. Neill may have some sense of that, but I think that that would be the output of the risk assessment, what proportion of the time does exposure lead to infection. I'm not sure that we have a good sense of that. Perhaps some of the speakers this afternoon will give us a little better sense of that too. Nick Daniels and Marianne Ross.

DR. MICHAEL JAHNCKE: Any other comments? Bob?

DR. ROBERT BUCHANAN: I guess one of my concerns would be that in the absence of some kind of good data characterizing how the oysters are handled once they are

harvested, and considering the -- you're almost restricted to using market data. That gets to be -- making the link between the ocean and the market becomes tenuous and it's going to have a high degree of uncertainty.

Certainly any data that could be acquired in that region would be particularly helpful in coming up with the best estimate of exposure possible. But in the absence of that, you would have to assume market data.

DR. MICHAEL JAHNCKE: I'm wondering if -- I know that these guidelines and things from ISSC have been very recent, but I'm wondering if some of the shellfish departments in the states may have some of that data. I'm not sure if the State of Virginia has some of that, but maybe some of the other states may have some -- I believe it would probably be limited, but they may have some data.

DR. MORRIS POTTER: This is Morrie Potter. I'd like to see if Chuck or Andy have any information that would be useful in that regard that they would like to add to their prepared statements. Andy DePaola is coming up.

DR. ANDY DEPAOLA: I'm always ready to add something. FDA did a study several years ago at the dealer level for vibrio vulnificus, the levels of vibrio vulnificus that were seen. The dealer, the harvester catches the oysters and they bring them to the wholesaler,

this is at the point where they are washed. Those levels were about the same as what we saw in the Gulf oysters in the retail study. I think this data suggests that most of the increases occur before processing. Or, in transport to the dealer and cooling down in the refrigerator. So that kind of limits part of it.

DR. ROBERT BUCHANAN: Certainly that's a very important piece of data since they suggest that the temperature control from the dealer on is fairly good.

DR. MORRIS POTTER: Dr. Neill?

DR. MARGUERITE NEILL: Peggy Neill. I don't know if this might have been covered first thing before I came in this morning. I think there is a slide which outlined harvest through to retail or consumption. Are there time frame ranges that exist for the different steps, and do we know anything about regional differences in those?

DR. MORRIS POTTER: I don't know that we do. I think the -- here around this table I think perhaps the question is, will they be able to build that into the risk assessment model so that at the end of the risk assessment we'll have some sense of how important the duration of each step is in the overall risk.

DR. MICHAEL JAHNCKE: Bill Watkins, you had a

question or a comment?

DR. ROBERT BUCHANAN: Mike, maybe we could bring the presenters up to the table so that they don't have to keep popping up and down from their seats.

DR. MICHAEL JAHNCKE: That's an excellent suggestion. Yes, if the presenters from this morning would please join us. Dane?

MR. DANE BERNARD: Dane Bernard. Any of the speakers, maybe you can enlighten me on what the fate of a vibrio is if it happens to encounter an oyster in the natural environment. Are we talking about just a pass-through? Does the oyster in fact break down vibrios? Is this just coexistence or do oysters use vibrios as an energy source? Does anybody even know, have any ideas, speculation?

DR. WILLIAM WATKINS: This is Bill Watkins, FDA Office of Seafood. It's my impression from all of the studies I've looked at and the results published, and some work that I've done, that during the warmer months when parahaemolyticus is prevalent and thrives it's difficult to find an oyster, and for that matter a clam, that does not have parahaemolyticus and a number of other vibrios associated with it. So looking at it from that standpoint I view the molluscan shellfish as part of -- having

vibrios is part of their normal flora during the permissive seasons. They grow very well, and as was indicated in previous studies, there's an output of vibrio vulnificus from oysters with the bacteria growing. Part of their normal flora.

That brings to questions what's the normal state of the oyster. I saw some work done years ago by Jeff Scott at National Marine Fisheries Service, he did not characterize it by species, but he did break it down into genus. He showed that with some of the oyster diseases that we see, I believe it was dermo and maybe perhaps MSX, with diseased oysters the flora of bacteria that populate them changes and there are increases in the levels of vibrios.

Thinking of an oyster reef that is being harvested actively, at any given time you might expect a certain percentage, perhaps real low, sometimes greater than low, oysters being diseased. Their health being compromised and therefore, their natural flora perhaps shifted. And, it might be those animals that are presenting us with a greater problem, it might not be a factor at all. Don't know.

I recall a question earlier about the Kanagawa phenomenon and how do we explain the one or two percent of

clinical cases where Kanagawa positive or TDH positive isolates were not obtained. How do we explain the fact that the TDH negative strains that were obtained are causing this illness? I think that might perhaps be an artifact of the methodology that we're using. You have to realize that we pick a number of representative colonies from the streak plates. Those streak plates come from the alkaline peptone water enrichment broth and those are inoculated with the fecal specimens from patients, or the patients' stools may be streaked directly.

But the causative strains may not have been found or picked or grown on the TCBS plates, and that may be what we're seeing. The negative strains that went and passed through the patient at the same time the causative strains were present. Of course, it's possible there are some other factors involved too.

DR. ROBERT BUCHANAN: Could we go back and revisit Dr. Potter's question about injured cells and how accurate your measurements of thermo resistance and acid resistance are? In those studies that you examined in preparing for this talk, did they take into account injury phenomenon so that they had the accurate D values or Z values or whatever? Or, are these values exaggerated in their effectiveness?

DR. WILLIAM WATKINS: I think it's fair to say the vibrio studies, from what I have seen, injured cells are rarely taken into account.

DR. ANDY DEPAOLA: We used two different methods. They both relied on initial steps of non-selective media. The DNA probe method, and this is with the vibrio growth data from Jan Gutch's work, we did direct plating to T-1 and 3, and we also did the FDA MPN procedure, where we inoculated alkaline peptone broth, which has only the selectivity of pH 8.5, which is optimal for vibrio parahaemolyticus. Usually injured cells are easier to recover in a broth than on a plate.

In her work we saw no difference between the MPN method and the plating method. I'm sure that some of the cells were injured and were not recovered by either method, and probably if we got down to it, we may even have some viable but non-culturable cells there. The ability of these that cause disease compared to the non-injured flora would be another issue.

DR. ROBERT BUCHANAN: Just as a general comment I'm interested in hearing more about the justification for ignoring the right-hand side of the post-harvest side of processing. It doesn't mean that I think that's a mistake, but I'm particularly interested in hearing, at

some point, from the epidemiologist about what is the extent of disease associated with shucked oysters.

DR. WILLIAM WATKINS: I can't answer that. I think we will hear that this afternoon. One thing we can say, I think, is that shucked oysters I do not believe have caused an outbreak, perhaps sporadic cases.

DR. ANDY DEPAOLA: I think there's some data from Washington, Chuck, isn't there? I recall a few cases where shucked oysters were implicated. I don't think we should totally ignore them.

DR. ROBERT BUCHANAN: I guess it was you, Andy, that showed a fairly busy slide of the different regions in terms of the levels that were present at market.

DR. ANDY DEPAOLA: Yeah, would you like to see that again?

DR. ROBERT BUCHANAN: I guess the question is, can you relate those levels at market to the incidents of disease in those different regions?

DR. ANDY DEPAOLA: That's really the goal of the retail study, was to be able to do that. When we originally began the study it was focused on vibrio vulnificus as the outbreaks. We started to plan the study several years ago before we had the vibrio parahaemolyticus outbreaks, and the reporting for vibrio

vulnificus is much more complete, we feel, as the primary, septicemia is more likely to be reported. There's about 40 cases per year, and we know what the various months of the year are, we know what the average number of cases are, and we can use those levels in market to see what level of exposure is related to illness.

Unfortunately, I'm afraid with vibrio parahaemolyticus it's much more under-reported and we don't know how under-reported it is. If we look at the incidents of illnesses, reported illnesses, I think that we can use that exposure data.

What I didn't show is -- what was presented there is the total vibrio parahaemolyticus population and we're not sure that that's really indicative of risk.

We are also going back and testing isolates for TDH genes to see what the incidents and quantity of pathogenic strains are. Maybe at that point we'll begin to get a handle. I think any kind of estimates of risk are going to have a lot of variability.

DR. ROBERT BUCHANAN: You also, in presenting that section, made a statement that I'd like to follow-up, because I think it impacts a lot on estimating risk. You said that oysters that were consumed in the different regions were largely home grown and home used. That is,

if you harvest oysters in Alabama, they're eaten in Alabama. If you raise them in New Jersey, you eat them in New Jersey. How strong of a statement is that? I mean, is that really the case? Is there not much interstate shipment of oysters?

DR. ANDY DEPAOLA: The particular states that we showed were all coastal states and they have a fairly large production. I think they were Washington and most of their oysters were home grown. In California there's not much production and they consume a lot of Gulf oysters there.

DR. CHARLES KAYSNER: They're the paramount of the west coast that's shipped out of state to the east. Probably not into the Gulf region.

DR. ANDY DEPAOLA: They tend to get shipped inland more is what we found, like to Denver and Chicago.

DR. ROBERT BUCHANAN: So there is a substantial amount of interstate shipment.

DR. ANDY DEPAOLA: A tremendous amount. These oysters travel a lot more than I do. They can be harvested in Texas, processed in Florida, and sent to New Jersey.

DR. MICHAEL JAHNCKE: Other comments from committee members? Yes, Cathy.

MS. CATHERINE DONNELLY: Just one question. Has anyone done any work on competitive exclusion as a type of prevention strategy in oysters?

DR. ANDY DEPAOLA: I'm not aware of any. One thing I think you have to realize, oysters, probably more than any other food we eat, they're not just a live animal. There's a whole ecosystem in there, all kinds of competitors, other vibrios, and the constant changes in salinity between tidal movements and everything give one organism a little bit of favor over another. I think nutrients are probably not a limiting factor. There's a lot of nutrients available, but there's tremendous phage populations.

The phages that we'll see in either the vibrio parahaemolyticus or the vulnificus often outnumber the strains a thousand to one. Not only does that maybe control their numbers, but it selects certain populations as what's going on in one oyster and the oyster right beside it could be dramatically different, because each one there's times when it closes is a closed system, and then when it opens, which is not a simultaneous -- not every oyster opens at the same time. The water that's moving through may have different things.

But, getting back to comparative exclusion, that

would probably have to be done under depuration and there have been some proposals to use phage with vibrio vulnificus.

A gentleman in Louisiana State University in New Orleans has had proposals in preliminary data where he was able to get some reductions using phage. But the problem with that is these phages are quite strain-specific, and as one strain is eliminated another strain may be favored.

DR. MICHAEL JAHNCKE: Bob?

DR. ROBERT BUCHANAN: I'd like to follow-up one piece of information. I think, Bill, you presented this morning, it was a bell-shaped curve looking at salinity and at the maximum level of growth that it was achieved. Do you see that same bell-shaped curve in vibrio levels in oysters if you were to take them from those different types of environments? Do you get higher or lower levels?

DR. WILLIAM WATKINS: Bill Watkins, FDA. I don't know the answer to that. We've -- there are many examples of laboratory data produced, testing various salts and salinities. I don't know of any of them that used oysters or clams to determine the levels based on salinity changes. Don't know that that has been done.

DR. ANDY DEPAOLA: Once again we have some information both from our laboratory studies and from the

retail study. The average salinity in oysters at retail, and the way we measured this was to take a drop of the manna liquor which we've seen reflects pretty accurately the salinity of the over-lying waters. It was about two-and-a-half percent or 25 parts per thousand. If there's any trend that we've seen with the environmental data there's a slight negative correlation between vibrio parahaemolyticus numbers and salinity.

What we've seen with vulnificus, as long as the salinity is above five and below twenty-five it has very little impact on their densities. Above that and below that they begin to decline.

DR. MICHAEL JAHNCKE: Other questions or comments?

DR. CHARLES KAYSNER: Chuck Kaysner, Food and Drug. I have a question for Andy. On the Ameri Pure process, which is a heat treatment as I understand, what temperature do they use and how long is that process?

DR. ANDY DEPAOLA: I believe it's the same as described in the paper. The internal temperature of the oyster, I believe, is 50 degrees Centigrade and is kept there for five minutes.

DR. MICHAEL JAHNCKE: Anyone else from the subcommittee?

DR. CHARLES KAYSNER: Chuck Kaysner again for Dr. Buchanan. I recently put together a table for a chapter which compiled D values for what I could find in the literature for vibrio cholera, vibrio parahaemolyticus, vibrio vulnificus. Unfortunately I didn't bring it with me.

A lot of that work was done with homogenates of crayfish, shrimp, those types of products. As I remember, there was only one D value for an oyster and it was probably a homogenate at 60 degrees, and I think we're looking at somewhere around ten minutes. But, I can get that information. I can get that out.

DR. ROBERT BUCHANAN: Bob Buchanan, FDA. Were they all pretty similar?

DR. CHARLES KAYSNER: Yes, uh-huh. When you look across the board the heat sensitivities between the three species seemed to be quite similar.

DR. ROBERT BUCHANAN: So that if you didn't have specific data for oysters there would be data based that you could use to estimate that in terms of processes using homogenates of shrimp or fish or whatever.

DR. CHARLES KAYSNER: I think so.

DR. ROBERT BUCHANAN: And it would be reasonable. Okay.

DR. MICHAEL JAHNCKE: Other questions, comments?

DR. MORRIS POTTER: Okay, this is Morris Potter. The non-committee participants in today's hearing have been sitting patiently listening to the events this morning. We will have scheduled time for non-committee participation this afternoon, but if there is anyone who would like -- anyone from the audience who would like to offer some information or make other comments now, we do have some time. Ken Moore?

MR. KEN MOORE: Ken Moore, ISSC. Bob Buchanan asked a question about information being available regarding temperature and how temperatures maintain at different levels. While I'm not aware of anything specific for parahaemolyticus, when we were conducting an assessment of the NM control plan that was adopted for vulnificus back in 1995, we did an assessment that gave us some data that is available. But, it is specific to vulnificus. But, it does offer some ideas about temperatures, I think both ambient and internal regarding different points and distribution.

DR. MORRIS POTTER: Ken, as a point of clarification, is that information available to the Office of Seafood so that it can be entered in?

MR. KEN MOORE: Yes.

DR. MORRIS POTTER: Other comments from the floor? Again, there will be another opportunity as the Federal Register said after lunch. But, if not, we will break for lunch now. It's 11:40, so we will return at 12:40. Thank you.

(Whereupon, a lunch recess was had in this matter.)

DR. MICHAEL JAHNCKE: Before we get the afternoon session started Mary Harris has some administrative information for members of the subcommittee and the NAC Committee.

MS. MARY HARRIS: I just wanted to talk to you just a little bit about travel. From what I'm hearing, I've heard some of the committee members have had some problems with making travel arrangements and what have you, or they've had trouble getting reimbursed for travel expenditures. In an effort to try and remedy some of these problems what we're going to do is, I understand that there are a couple committee members that may be leaving early. So between the hours of 5:00 and 6:00 today and tomorrow Chevon Morris and myself will be sitting outside in the registration area and we'll be glad to take down any comments or problems that you've had and try and look into them and see how we can, hopefully

remedy those, and also assist you in filling out your travel vouchers. What we're going to do is have you sign travel vouchers before you leave and then you'll take back a copy, and if you have any additional changes you can fax them to Chevon or myself and we'll fill in the changes. Then we go ahead and just send them off to have you reimbursed for them, to hopefully get you reimbursed a little bit quicker.

It will be today between 5:00 and 6:00, tomorrow between 5:00 and 6:00, and then on Friday we'll be there between 12:00 and 3:00 to answer any questions, or to assist you in filling out your vouchers. Okay? Thank you.

DR. MICHAEL JAHNCKE: Thank you, Mary.

DR. MORRIS POTTER: Welcome back for the second half of the vibrio parahaemolyticus risk assessment public hearing. At this point in the hearing we would like to provide a more formal opportunity for non-committee members to comment on this morning's proceedings, or deliver any other public comments that they would like to have entered into the record.

There's also a written record for folks who would like to make comment. That record is open for awhile. I'm told June 30. So anyone in the audience who

would like to make a comment? Alright, in that case I will turn the program back over to Mike Jahncke and we'll proceed with presentations on the risk assessment.

DR. MICHAEL JAHNCKE: Thank you, Morrie. Our next presenter is Dr. Marianne Ross, and she will be speaking about epidemiology in the public health module.

DR. MARIANNE ROSS: Good afternoon. I have the task of talking right after lunch so everybody is nice and sleepy, huh.

I am Marianne Ross. I'm delighted to be here today. I will go as long as I can with this voice. I'm here to present the epidemiology of vibrio parahaemolyticus infections associated with the consumption of raw molluscan shellfish in the United States.

On the agenda for my section, as you can see, I'll go through a very brief introduction. I'll talk about the methods of our data collection. I'll get into some definitions of some terms that I'll be using throughout the section.

I'm going to talk about two different types of data. One is case series data. The other is outbreak data. For each of those I'm going to give you the most illustrative examples that I had, to explain those a

little further. So for the case series I'll go into a Gulf Coast vibrio surveillance survey and the first year's results.

Then I'll go into a case series that was done in Florida between 1981 and 1994.

When we get to the outbreaks I'll concentrate on one outbreak in particular, and that is the Pacific Northwest outbreak in 1997.

Then I'll go through some of the limitations of our data, and finally I'll end up with a summary of the entire literature search. That will include case series and outbreak data.

Usually vibrio parahaemolyticus presents clinically as gastroenteritis. That usually is a mild duration and not as severe as septicemia. But, septicemia can occur and can be life-threatening.

Persons with septicemia often do have underlying medical conditions.

Now, the methods of our data collection. We did a Pub Med/Medline search. We limited that to English language peer-reviewed literature. We also limited that to occurrences within North America. We did not put a time restraint on our data search. So subsequently, our data spans from 1972 to 1998, and from that search we were

able to gather 11 peer-reviewed articles to explore further.

I'll go over some definitions at this point, things that I'll be referring to throughout the section.

Raw molluscan shellfish refers to either raw oysters, mussels or clams. However, the data that I have for this section primarily talks about the consumption of raw oysters. Very little information do I have on persons consuming raw clams and none for persons consuming raw oysters (sic). Just to give you an idea of what I'll be concentrating on. As I said, I'll talk about two different types of data.

A case series is a study of sporadic cases over a period of time, and usually a case series is limited to a certain geographical area, as we'll see when I get into that section later on.

An outbreak is defined as the occurrence of two or more cases of a similar illness resulting from consumption of a common food source.

When I get into the clinical history and the clinical presentations of vibrio parahaemolyticus I'll talk about two distinct syndromes that are observed with vibrio parahaemolyticus. Those are gastroenteritis and septicemia.

Gastroenteritis is an illness that's characterized by vomiting, diarrhea, abdominal cramps, and the organism would be isolated from a person's stool sample.

Septicemia, on the other hand, is an illness that's characterized by fever, hypotension, and hypotension is usually defined as a systolic blood pressure of less than 90. The vibrio organism would be isolated from a person's blood, as opposed to a stool sample. Just bear in mind that both of these syndromes can occur as a result of consumption of raw molluscan shellfish.

These next three slides I'm going to concentrate just on the case series data, and from our literature search we found that there were 7 case series.

First of all, I'll give you an idea of where these case series occurred. So this map is for vibrio parahaemolyticus case series. It gives you the location, the year or years that the series took place, and in parentheses the number of cases that were effected.

I just wanted to point out that in Florida there were actually two case series done during that time, with a total of 186 persons effected.

What I will do next is concentrate on that Gulf

Coast area. I'll talk about a very unique system of vibrio surveillance.

This is called the Gulf Coast Vibrio Surveillance Program. This is, as I said, a very unique regional Vibrio Surveillance Program. It began in 1989, and as you can see, there are four states that participated in this program. They are listed there: Alabama, Florida, Louisiana, and Texas. Investigators in local and county health departments gathered data for all persons for whom vibrio isolates were identified. Those vibrio isolates can be identified either from laboratories or individual physicians, or hospitals.

Information is collected onto a Standardized Vibrio Illness Investigation Form. That form contains information such as clinical history with the person they have presented with clinically, any underlying medical illness that a person may have, any medications that a patient was on. It also contains epidemiologic information, and in particular it contains information on seafood history, a seafood consumption history in the week prior to illness.

Once this information is gathered on these Standardized Vibrio Illness Investigation Forms they are then reported to CDC, where they do further analysis and

compilation.

What I'll do now is I'll concentrate on the first year of the results of this Vibrio Surveillance Program. And as I said, that was 1989. Just to let you know, information has been gathered and compiled since that time, but has not been published to date. So, I'll concentrate on the published results of the first year.

As you can see, there were a total of 85 vibrio isolates. Of that 85, 27 persons were identified with vibrio parahaemolyticus.

Of those 27, as you can see, 26 presented with gastroenteritis. One presented with septicemia.

Of those 85, total persons with all vibrio species, 69 percent of those consumed raw oysters. Unfortunately, I don't have information that is broken down per species as to how many persons ate raw oysters. So, I'll remind you that this is for all vibrios.

Oyster-associated infections were found to occur all throughout the year, but there was a peak that occurred in October.

That was the first year results of the Gulf Coast.

Now, what I'm going to do is move on to another case series, and this case series was between 1981 and

1994. It took place in Florida. Bear in mind that this a case series related specifically to raw oyster consumption.

Culture-confirmed case reports of vibrio illnesses are reportable in Florida to the Florida Department of Health and Rehabilitation Services. And again, Standardized Vibrio Illness Investigation Forms are used. Information is gathered by local and county health departments.

These case reports were then reviewed to determine of the epidemiology of, as I said before, specifically raw oyster-associated vibrio illnesses. All cases in this case series had a history of raw oyster consumption in the week prior to illness. These persons presented either with gastroenteritis or septicemia.

They also determined that the average annual incidents of raw oyster-associated illness from vibrio species was 10.1 per million. That is among raw oyster consuming adults. An adult in this case was considered to be anyone over the age of 17.

The annual incidents of fatal raw oyster-associated illness from vibrio species was 1.6 per million. Just to give you an idea of where some of this information came from to get the denominator data, there

was a survey completed in Florida in 1988. It was a Behavioral Risk Assessment Survey. Participants in that survey were asked questions such as: "Do you consume raw oysters? If so, how often?" So that's where some of this information came from.

I'll give you some of the results of this case series. As you can see, vibrio parahaemolyticus infections accounted for about 23 percent of all vibrio illnesses that were gathered during that time.

Of the 77 persons who were identified with vibrio parahaemolyticus 68 presented with gastroenteritis, whereas 9 presented with septicemia.

This shows the number of cases and the months that they occurred for vibrio parahaemolyticus infections. As you can see, as in the last case series, cases occurred all throughout the year, but in this case series there was a peak in September.

Again, from the slide we saw before, there were 77 persons identified with vibrio parahaemolyticus infections. Of those 77, 37 were hospitalized. The majority of those were hospitalized for gastroenteritis. Eight were hospitalized for septicemia. There were four deaths, and it's interesting to note that all of those deaths were associated with septicemia.

Those were two examples of case series data.

Now I'm going to switch gears and I'm going to concentrate for the next few minutes on strictly outbreak data.

From our literature search we found that there were four published outbreaks. This map shows you again the location of the outbreaks, the year that the outbreak occurred, and in parentheses how many persons were affected.

As you can see, there were two very recent outbreaks on either coast. Just to let you know, Dr. Nick Daniels will be talking about an outbreak that occurred in Galveston Bay, which is not included in this because I limited mine to strictly published literature.

Also interesting to note is that prior to that 1981 outbreak, like in the late seventies or early eighties, vibrio parahaemolyticus infections were thought to occur mainly along the Atlantic Seaboard or in the Gulf Coast area. But, as you can see, now we've had to expand our thinking into the Pacific Northwest region when we talk about vibrio parahaemolyticus now.

During this time, on the basis of increased illness reports either from local and county health departments or from ill persons themselves, public health

officials in that Pacific Northwest area were quickly alerted to an outbreak problem and prompted this investigation.

The dates of onset of illness ranged from May to December, and the peak was in July and August in this outbreak. There were 209 persons affected.

Just to give you some of the clinical features. The median age was 39 years and it had a range of 12 to 85 years. Most of the persons were male. The symptoms predominately were diarrhea and abdominal cramps. But, as you can see, nausea, vomiting, fever, and also bloody diarrhea can occur, but they occurred less frequently.

Again, I said there were a total of 209 cases effected. Two patients were hospitalized. There was one death and that death also was associated with septicemia.

Most cases did not report having any underlying illnesses. As a matter of fact, only 17 persons of the 209 reported having underlying illness, but that illness was not defined further. So we don't have any specific categories for you.

That was an example of the largest published outbreak, which took place in the Pacific Northwest in 1997.

Now what I'm going to do is I'm going to combine

the literature search to include outbreak data and case series data.

As I said, there were 7 case series and 4 outbreaks that we found during our literature search. There were a total of 270 persons affected in the case series and 250 persons affected during outbreaks. So we have a total of 520 cases of vibrio parahaemolyticus.

For the case series, as we saw, the range of infection was all throughout the year with certain peaks in September to October.

For the outbreaks the range of infection was from May to December and the peak there was August/September. A total of 520 persons affected, and as you can see, 97 percent of those were affected with the syndrome of gastroenteritis, and 14 persons were affected with septicemia. 43 persons were hospitalized, and again, the majority of those hospitalized for gastroenteritis.

Interesting to note that 12 of those 14 persons with septicemia were hospitalized. The duration of hospitalization ranged anywhere from one day to thirty days, with a mean of about five days.

Continuing on, and again this a combination of the case series and the outbreak data. The age range for those persons affected was from 9 months to 91 years, with

a mean of 38 years. That 9 month old was verified. The investigator who was working on that case in Florida contacted the parents, and indeed, the father had fed that child raw oysters.

The majority of cases were males, White males. A total of 9 persons died, and all of those deaths were related to septicemia.

We found that the incubation period ranged anywhere from 12 hours to 96 hours. The number of raw oysters consumed had a very wide range, anywhere from one oyster to 109 oysters, with a median of 12 oysters.

Just a little footnote, the 109 oysters, I'm not sure as to whether that was consumed over one meal or if that was consumed over a period of three days during a convention. Nonetheless, the total was 109.

As I said, most cases typically present clinically with gastroenteritis. Those folks who do present with gastroenteritis usually experience diarrhea and abdominal cramps, but other symptoms can occur, but they occur less frequently.

Septicemic patients, on the other hand -- and bear in mind, septicemia we referred to as having a fever or having hypotension, and septicemic patients are also those patients who are more likely to die from vibrio

parahaemolyticus.

Septicemic patients were often reported to have underlying medical conditions, and some of those medical conditions that we found in the literature ranged from cancer, diabetes, liver, kidney, and heart disease.

That was the summary of the outbreak and case series data.

Now I'll get into some of the limitations of our data. The first limitation is that data quality varied. That may be for several reasons, but bear in mind that this information was gathered over a period of 26 years. Certainly during that span of time reporting and diagnostic procedures may certainly have changed. That effected our data quality.

Because vibrio parahaemolyticus tends to present as gastroenteritis, which has a milder severity and a relatively short duration, under reporting is thought to occur.

Another limitation is that the details of the clinical symptoms and details of risk factors, especially those risk factors associated with a person's underlying illness or seafood history, are not uniformly and routinely reported throughout the literature.

Finally, much of the information in the

literature is presented for all vibrio species together, which makes it rather difficult to extract information related specifically to vibrio parahaemolyticus.

A few conclusions. Sporadic cases of vibrio parahaemolyticus occur. They are reported by several states throughout the U.S, but primarily reported by Gulf Coast states.

In addition to sporadic cases, outbreak cases do occur, and we have seen those occurring very recently on either coast.

Most cases of vibrio parahaemolyticus do present as gastroenteritis, which is usually mild and has a lower case fatality rate. However, bear in mind that life-threatening septicemia can occur, especially in those persons with underlying illnesses.

So what we've done is, I've gone through the methods of our data collection. Gone through several definitions. I've talked about two specific types of data, and that was case series and outbreak data, and gave some examples of each.

Then I gave a combination of the outbreak data and case series summary. Talked about some of our limitations, and finally some conclusions.

At this point I'd be happy to answer any

questions you may have.

DR. MICHAEL JAHNCKE: Questions from members of the subcommittee?

DR. MORRIS POTTER: Bear in mind that after the three EPI presentations we'll get the presenters at the table and be able to have another shot at them.

DR. MARIANNE ROSS: Comforting. Thank you.

DR. MICHAEL JAHNCKE: Yes, Dane?

MR. DANE BERNARD: Thank you. Dane Bernard. Thank you for your presentation. Very nice summary.

I notice from the table that's in the background material that you provided, which is one of your slides, that we have outbreaks in sporadic cases associated, as you said, with not only vulnificus and parahaemolyticus, but hollisae mimicus. The table seems to break it out, but how much confidence do we have in this? Is this going to complicate the job of doing a risk assessment solely on vibrio parahaemolyticus.

There was also a question from behind me here about whether the 109 oysters may have been consumed by the nine-month old, but I think that's a spurious thing we can disregard here.

But, your analysis of that table, if you would, please.

DR. MARIANNE ROSS: If I understand this correctly, the table has species identified and then how many persons had gastroenteritis and how many had septicemia. That actually, in my way of thinking, makes it a little easier for us, because we can pick out the parahaemolyticus infections. The ones that I mentioned in some of the limitations gave information on all vibrios together and didn't pull that out, and that is definitely a limitation. The articles that did verify and break down the species made it a lot easier. Others I had to give just very general information on vibrio species together. Did that answer --

DR. MICHAEL JAHNCKE: Other questions? Bob?

DR. ROBERT BUCHANAN: Bob Buchanan. See, I did remember to say my name. Do you have any estimate at all of the number of people that had underlying conditions that did not show septicemia?

DR. MARIANNE ROSS: The only information I have, it's very limited, on underlying illness is I have one article out of that literature search that tells me for four persons, of the 14 with septicemia. I have four persons who I know exactly what they had in terms of clinical symptoms, in terms of their underlying illness, and their consumption. That's very limited. You brought

up a good point that the information is usually lumped in there. The article will say, we had two persons with septicemia, and no more information as to whether those persons had underlying illness or not, or as a matter of fact what their clinical outcome was.

So, I'm afraid I don't have any more specific information.

DR. ROBERT BUCHANAN: As a follow-up on that have you given any thought at all on what value you're going to be using for the portion of the population that is at risk in terms of increased susceptibility to septicemia?

DR. MARIANNE ROSS: Actually in my section I have not been able to assess that at this point, but that is definitely something we are going to have to address, determining who is at risk and of those persons what are the underlying illnesses associated with the risk. I don't have an answer for that at the moment though, but that is one of the major tasks that we will have.

DR. MICHAEL JAHNCKE: Dane?

MR. DANE BERNARD: Thank you. Dane Bernard. You gave a rate of potential -- let's see, there was mortality, there was a mortality prediction or an estimate and an infection estimate per million of oyster-eating

population.

DR. MARIANNE ROSS: Right.

MR. DANE BERNARD: Do you have an estimate of how many millions of raw-oyster eaters that we're dealing with or not?

DR. MARIANNE ROSS: I believe Mike DiNovi may have that in, not the next section, but the section after that, on consumption. I don't have that information, but I believe Mike has consumption information coming up.

DR. MICHAEL JAHNCKE: Other questions? If not, thank you, Dr. Ross. Very nice presentation.

DR. MARIANNE ROSS: Thank you.

DR. MICHAEL JAHNCKE: Our next speaker this afternoon who will be speaking about the Gulf Coast outbreak is Dr. Nicholas Daniels.

DR. NICHOLAS DANIELS: Good afternoon. I'd like to present an overview of an epidemiologic investigation of an outbreak of vibrio parahaemolyticus in Galveston Bay, Texas during the summer of 1998, as well as present clinical and epidemiologic features of both sporadic V.P. cases and outbreaks.

A free-borne transmission of V.P. was first identified in 1950 in Japan during an outbreak investigation that found an infection was associated with

eating sardines. 272 persons became ill and 20 died.

The first confirmed outbreak of V.P. in the U.S. occurred in 1971 and was associated with the consumption of steamed crabs from Maryland.

V.P. causes three distinct syndromes of clinical illness, which includes gastroenteritis, the most common, wound infections, which occur after exposure of abraded skin to warm sea water or raw shellfish products, and septicemia in persons with chronic underlying medical conditions such as diabetes or liver disease.

Between 1973 and 1998 there were 40 outbreaks of V.P. infections in 15 states and Guam reported to CDC, resulting in 1064 illnesses.

Many of these outbreaks occurred during the warmer months with 80 percent occurring between April through October. The median month of occurrence was July.

During these reported outbreaks the median attack rate was 50 percent. It ranged from 3 to 100 percent. The median incubation period was 17 hours. The median number of ill persons involved in these outbreaks was 8, and the median duration of illness was 2.4 days.

Food vehicles in all of these outbreaks were seafood, and seafood was eaten raw in 15 or 38 percent of these reported outbreaks.

12 or 30 percent of the 40 V.P. outbreaks reported were reported in 1997 and 1998, suggesting a resurgence of this pathogen. Most V.P. outbreaks have occurred in the western states. Although last year was the first year since 1982 that these outbreaks were reported from the northeast and southern harvest sites.

The higher risk of vibrio infection during the warmer months is evident from this graph, which shows 345 sporadic V.P. infections from Gulf Coast states, Florida, Alabama, Louisiana, and Texas by month between 1988 and 1997. As you can see, most sporadic infections have occurred between the months of April and November.

All syndromes of V.P. infection were more common in the warmer months, with all septicemia cases occurring during May to November. Septicemia cases are on top there.

Of the 345 sporadic V.P. infections reported through passive surveillance between 1988 and 1997, 202, 59 percent, presented with gastroenteritis. 118, or 34 percent, had wound infections, and 17, or 5 percent, had septicemia.

Eight other infections were reported, including ear, eye, urinary tract and peritoneal infections.

As you can see, a high percentage of people with

gastroenteritis and with septicemia presented with bloody diarrhea suggesting that only the most severe cases actually come to medical attention.

Among the 97 patients with sporadic V.P. infection and known food histories 83, 86 percent reported eating raw oysters in the week before illness. Of these 70, 84 percent had gastroenteritis. Ten, 12 percent presented with septicemia, and three, or four percent presented with wound infections. Among 11 patients with septicemia and known food history, ten, 91 percent, had consumed raw oysters.

For sporadic V.P. infections 156, or 45 percent of persons were hospitalized and 119, or 34 percent of persons reported having a pre-existing illness. Of the 301 patients whose survival was reported 12, or 4 percent of persons died as a result of their infections. Among the 12 deaths 10 or 83 percent had known pre-existing medical conditions, including alcoholism, liver disease or diabetes. Of the five patients who died with information on food exposures all had eaten raw oysters.

V.P. is natural inhabitant of estuarine and marine environments. It is also a halophilic or salt loving, gram negative bacterium whose growth is promoted by high salt concentrations and warm water temperatures.

A selective agar media, TCBS, is often necessary for stool specimen isolation. Isolates can be sub-typed by serotyping and through post fill gel electrophoresis, PFGE.

Some strains are considered non-pathogenics since they do not cause illness in humans. Therefore, V.P. can be sub-grouped on the basis of pathogenicity. The presence of the thermostable direct hemolysin gene and the thermostable direct related hemolysin gene correlate with pathogenicity in humans.

In environmental surveys greater than 95 percent of isolates collected from persons with thermostable direct hemolysin are positive. Although less than one percent of isolates collected from the marine environment or food are thermostable direct positive. So greater than 95 percent are actually positive in clinical specimens.

These surveys suggest that the majority of V.P. found in the environment and in food is non-pathogenic.

On June 15, 1998 the Texas Department of Health was notified of an outbreak of gastroenteritis among patrons of two seafood restaurants. V.P. was isolated from stool cultures from two ill patrons. Interviews conducted with restaurant patrons demonstrated that illness was associated with consumption of raw oysters.

Oyster tags implicated a Galveston Bay harvest site as the source of contaminated oysters. Oyster beds were closed to harvesting on June 26.

T o enhance case ascertainment, the Texas Department of Health established a toll-free hotline and requested through a press release that ill persons call the health department to report gastroenteritis after eating seafood.

To further increase identification of suspect cases a memo was sent to the Texas Regional Health Districts, Hospital Infection Control Practitioners, and state and territorial epidemiologists to notify them of the outbreak and request that they contact Texas about outbreak-related, suspect, or culture-confirmed cases.

CDC was invited to assist with the ongoing investigation.

For surveillance purposes a suspect or culture-confirmed case in Texas was defined as a person with watery diarrhea with onset within 24 hours after eating seafood between May and July of 1998.

In other states suspect or culture-confirmed cases were defined as watery diarrhea within 24 hours after eating oysters traced to Galveston Bay.

Approximately 700 persons contacted the Texas

Department of Health hotline. Illness was reported in Texas and 12 other states. There were at least 416 persons who met our case definitions. In Texas there were 365 suspect and 31 culture-confirmed cases. Of those 93 percent reported that they ate raw oysters.

Cases from other states included 42 suspect and 78 culture-confirmed cases, all of whom ate raw oysters.

This map shows the distribution of cases in the U.S. As you can see illness occurred in 13 states, including Massachusetts, Tennessee, Colorado, Georgia, and California.

This graph shows the epidemic curve of the outbreak by date of illness onset between May and July of 1998. This outbreak was one of the largest V.P. outbreaks ever reported in the U.S. As you can see, case onset dates range from May 31 through July 4. Culture-confirmed dates are shown here in yellow. Also shown in this graph is the date the first case was reported to Texas Department of Health on June 15, sixteen days after the first illness onset, and when oyster harvesting ceased on June 26.

The predominant symptoms and signs among the 296 cases in Texas included diarrhea, which was part of our case definition, abdominal cramps, and nausea.

The median age of ill persons was 42 years. 59 percent were male, with a median duration of illness of five days. Fourteen or four percent of patients were hospitalized and there were no deaths reported. 110, 37 percent of ill persons sought care for their illness.

To further epidemiologically characterize this outbreak we conducted two restaurant cohort studies. We identified two cohorts of persons with more than ten persons who had eaten at an event at a restaurant in which at least one person had become ill and called the health department to report illness. We contacted persons from each of the cohorts and asked that they provide names and telephone numbers of all persons that had eaten with them at the event. Cases were defined as water diarrhea starting within 24 hours after attending the event.

These events occurred at restaurants A and B. One event at restaurant A involved a family member of 15 members who had eaten at the restaurant. The other event at restaurant B was a group of 30 persons of whom 15 were contacted. Looking at food-specific attack rate for eating raw oysters in the restaurant, A cohort, two of two or 100 percent of ill persons ate raw oysters, compared to two of 13, 15 percent of well persons.

In the restaurant B cohort eight of eight ill

persons ate raw oysters, compared to one of seven, 14 percent of well persons. The median number of oysters eaten by ill and well persons in these cohorts was five oysters.

The risk of becoming ill did not correlate with eating increasing numbers of oysters. One person became ill after consuming only one oyster.

Interestingly, eight of ten, or 80 percent of persons who became ill reported no underlying illness. I would like to emphasize this finding that these V.P. infections occurred in predominantly otherwise healthy persons. This is in sharp contract to vibrio vulnificus which effects primarily persons with chronic underlying illness.

What do the results of these cohort studies tell us about the knowledge, attitudes, and beliefs of oyster consumers?

In our cohorts, among the 13 respondents who consumed raw oysters 77 percent were aware of some health risks associated with consuming raw oysters, as well as the seasonality of vibrio infections.

However, 64 percent did not believe that they were at risk. Rationales given were:

One, I've eaten oysters all my life and I've

never been sick before, and;

Two, the perception is, the government allows restaurants to serve oysters, they must be safe.

What does this survey tell us? A high percentage of consumers knew about the health risks associated with eating raw oysters, but thought that they were not at risk.

Trace-back investigation was facilitated by oysters being tagged with identifying information. Therefore, if properly filled out and tags were still available oysters eaten by a cluster at a retail outlet could be traced to a wholesaler, to harvester, back to specific lyse sites.

Trace-back information was obtained from 101 or 24 percent of the 416 cases. These trace-backs implicated 20 or 67 percent of the 30 harvest sites in Galveston Bay as the primary source of oysters for persons who were sick. All five harvesters in Galveston Bay in operation during the outbreak period were implicated.

The harvesters sold to 25 wholesalers to distribute to retail outlets. Approximately 1.5 million oysters were harvested from Galveston Bay during the outbreak period, May 27 through June 24. Since the median number of oysters eaten by oyster eaters in our restaurant

cohorts was five, and attack rate among the oyster eaters was 71 percent, it is estimated that up to 300,000 persons may have been exposed to oysters during this outbreak, and tens of thousands of people may have become ill.

This graph shows the oyster harvest dates by oysters eaten by all persons in Texas. As you can see, the dates of implicated harvest range from May 27 through June 24. No outbreak related illnesses were reported after oyster beds were closed on June 26.

All clinical isolates in Texas were confirmed at the Texas State Public Health Laboratory. Clinical and oyster isolates were subtyped by serotyping and PFGE, as well as tested for virulence genes.

All of the clinical isolates of V.P. tested were serotype 03:K6 and were thermostable direct molluscan gene positive. PFGE of the clinical isolates were indistinguishable. Oyster isolates contained multiple PFGE patterns, but none of the oyster isolates harvested from implicated sites in Galveston Bay matched the outbreak PFGE pattern.

During the outbreak at Texas Department of Health oysters harvested from Galveston Bay contained V.P. with a median level of 15 V.P. organisms, most probable number MPN per gram of oyster meat and ranged from 3 to

4600 MPN per gram by the multiple tube fermentation method.

Extensive testing of oysters harvested from Galveston Bay, in the one to two months following the outbreak, the FDA found three isolates which were positive by TDH gene probe, thus indicating human pathogenicity. But none were reportedly 03:K6.

This means that were low counts found in oysters during the outbreak and highlights the difficulty in finding pathogenic V.P. using current microbial testing techniques that may not be sensitive enough to detect pathogenic V.P. at low levels in the environment when most V.P. in the environment is non-pathogenic.

What do we know about 03:K6 serotype? V.P. 03:K6 was first detected among strains from travelers in Southeast Asia at a quarantine station in Japan beginning in 1995.

In 1996 the same 03:K6 clone emerged as the dominant V.P. strain to cause V.P. illness in India. Currently it has become a common outbreak strain in Asian countries. The Galveston Bay outbreak identified 03:K6 for the first time in the United States.

Galveston Bay and Asian 03:K6 strains showed distinct but closely related patterns by PFGE, suggesting

that these strains may have been derived from the same clone, but may have genetically evolved over time.

There are some subtle differences here on the bottom.

This slide shows V.P. serotypes found in clinical specimens during outbreaks invested by CDC over the years. Of note V.P. serotypes 04:K12 and 01:K56 have repeatedly been isolated from the Pacific Northwest.

In 1998 03:K6 emerged in Texas and in New York.

The Galveston Bay outbreak investigation left several remaining questions. Where did this virulent strain come from and how did it get into Galveston Bay? Why did it occur during the summer of 1998?

In an attempt to explain some of these remaining questions we set forth to explore some possible hypotheses.

A possible answer to the question of how did this new strain get into the U.S. is that it may have been introduced through ballast water, which is water loaded on ships for stability going through the Houston Ship Channel to the Port of Houston, going right through the oyster harvest sites.

The fundamental problem regarding ship's ballast is that for most cargo ships to operate safely they must

carry substantial quantities of ballast water if they are not carrying cargo. Cargo ships often carry millions of gallons of water, of ballast during a voyage. Ships usually take on ballast from the body of water in which the ship originates. Having taken water on board it is normally retained until the ship is about to load cargo, at which point ballast water is discharged.

This is ballast water actually being discharged from a ship. During deballasting organisms from the point of origin may be introduced into the loading port. Therefore, probably ships discharging ballast water in the Houston Ship Channel or the Port of Houston may have been responsible for introducing V.P. 03:K6.

Data obtained from the Immigration and Naturalization Service show that between October, 1997 and June, 1998, 15 ships with Asian countries as their last port of call entered the Houston Ship Channel. This is not the first instance that ballast water has been suspected as a vehicle for non-indigenous organisms into U.S. waters.

For instance, in 1991 ballast water was suspected as a vehicle for transporting the South American toxigenic vibrio cholera 01 strain into Mobile Bay, Alabama. Testing of ballast water from several South

American ships docked in Mobile Bay confirmed the toxigenic strain.

To explore that prothesis of whether favorable environmental conditions allow the emergence of this pathogen we did an environmental survey of monitoring sites. We randomly selected seven, or nine percent of the Texas Department of Health existing 76 monitoring sites for environmental conditions in Galveston Bay. We compared water temperature and salinity levels before and during the outbreak with environmental data recorded over the previous five years.

Comparison of mean surface water temperatures during May and June of 1998 with mean surface water temperatures during the corresponding months in the previous five years found a significant difference in mean values.

During May water temperatures were 81 degrees Fahrenheit compared to 76 degrees Fahrenheit for the previous five years.

In June 85 degrees Fahrenheit compared to 83 degrees Fahrenheit for the previous five years.

These mean values were significantly different during both May and June of 1998.

Comparison of mean salinity concentrations were

also significantly higher during the months of May and June, 1998, compared to the corresponding months in the previous five years. As a result of significantly less rainfall during April and May preceding the outbreak, low rainfall .59 inches during April and .02 inches during May, caused extreme draught conditions in Texas and markedly increased salinity levels in Galveston Bay.

During May salinity levels were 18.3 parts per thousand compared 8.4 parts per thousand for their previous five years.

During June 21 parts per thousand compared to 9.1 parts per thousand for the previous five years.

These mean values were significantly different. Therefore, it is likely that environmental conditions such as elevated water temperatures and increased salinity levels, which we know promote the growth of V.P. organisms, may have contributed to this outbreak.

What lessons have we learned from the Galveston Bay outbreak? Current regulations allow the sale of oysters for raw consumption if there are less than 10,000 V.P. organisms per gram of oyster meat. During the Galveston Bay outbreak the median level of organisms was 15 MPN per gram of oyster meat at the Texas Department of Health Laboratory.

Current Texas regulations allow up to ten hours from harvest to refrigeration. During this outbreak the median time from oyster harvest to refrigeration was 5.5 hours within current regulatory limits.

Since the doubling time of V.P. is as short as nine minutes if left at ambient temperatures and the infective dose of V.P. is between ten-to-the-fifth and ten-to-the-seventh organisms, oysters left for short periods of time at warm temperatures could have led to a rapid proliferation of V.P. to infectious levels.

What are some potential prevention strategies? Cooking oysters could prevent illness by killing vibrio. Unfortunately, cooked oysters obviously taste different and few oyster eaters prefer cooked oysters. So alternative strategies are needed.

Oyster harvesters could ice or refrigerate oysters immediately after harvesting and keep them at low temperatures until consumed to reduce multiplication time of V.P.

Industry could develop technologies to eliminate or reduce vibrio contamination of shellfish. There have been some attempts in this regard, but the effectiveness has not been evaluated, and if vibrio is not eliminated it could grow to infectious levels.

An important additional safeguard may be the monitoring of water temperatures and perhaps salinity at harvest sites.

In conclusion, during the past several years the number of reported outbreaks of V.P. infection has increased steadily, with a sharp rise in 1997. Pathogenic V.P. 03:K6 not seen before in 1995 in the world is now emerging as a cause of gastroenteritis.

During the summer of 1998 V.P. 03:K6 clone was detected in the U.S. and caused severe watery diarrhea in previously health persons who ate raw oysters.

Furthermore, favorable environmental conditions such as elevated water temperatures, low rainfall producing extreme draught conditions in the preceding months leading to markedly elevated salinity levels may have contributed to this outbreak and facilitated the emergence of V.P. 03:K6.

Thank you.

DR. MICHAEL JAHNCKE: Questions for Dr. Daniels? Yes, Catherine.

MS. CATHERINE DONNELLY: Yes, Cathy Donnelly. In your cohort study you identified some patients with underlying illnesses. Can you explain what those underlying illnesses were?

DR. NICHOLAS DANIELS: Most of them had problems with their gastrointestinal tracts. I think most of them were on H-2 blockers or antacids primarily.

DR. MICHAEL JAHNCKE: Yes, John.

MR. JOHN KOBAYOSHI: John Kobayoshi, Seattle, Washington. Is chlorination or some other form of disinfection of ballast water a consideration?

DR. NICHOLAS DANIELS: It's a consideration, but the volume of the water could be a problem. That's what I've heard from people that are in the ship industry. It's millions of gallons of water, and to chlorinate that could be extremely difficult. I think they've tried radiation or sort of different kinds of technologies, but chlorination has been suggested, the practicality, I'm not sure of.

DR. MICHAEL JAHNCKE: Bill?

MR. WILLIAM SVEUM: Bill Sveum. You had a chart, and it showed outbreaks. There was a huge number on cruise ships, let's say 15 years ago, and then it disappeared. Can you correlate that to is it temperature control, is it better sanitation? Was there something that significantly changed to cause that drop-off?

DR. NICHOLAS DANIELS: Most of the cruise ship outbreaks were related to shellfish. Back in the

seventies and eighties I believe they used to have shellfish platters when people go on board. Subsequently, after many of those outbreaks, they stopped doing that.

DR. MICHAEL JAHNCKE: Yes, Bob?

DR. ROBERT BUCHANAN: Bob Buchanan. In your data on the outbreak you listed 14 people being hospitalized.

DR. NICHOLAS DANIELS: For the Texas outbreak?

DR. ROBERT BUCHANAN: Yes.

DR. NICHOLAS DANIELS: Yeah.

DR. ROBERT BUCHANAN: How many of those people had septicemia?

DR. NICHOLAS DANIELS: In the Texas outbreak there was only one person that had septicemia. All other were stool isolates and were gastroenteritis.

DR. MICHAEL JAHNCKE: Dane?

MR. DANE BERNARD: Thank you, Dane Bernard. 03:K6, is there any -- other than the fact that it showed up in 1995 and you hadn't seen it before, does it appear to have any characteristics that make any worse than other strains that we've seen before?

DR. NICHOLAS DANIELS: I can't say we've looked at that. I don't know if some other labs have, with the FDA. It's TDH positive. A lot of strains are TDH

positive. It's urease negative. It's different from the Pacific Northwest, but nothing to distinguish it as being more virulent per se.

DR. MICHAEL JAHNCKE: Yes, Margaret.

DR. MARGUERITE NEILL: Peggy Neill. Do you have any simultaneous data on vulnificus infections?

DR. NICHOLAS DANIELS: Not with me.

DR. MARGUERITE NEILL: From Texas?

DR. NICHOLAS DANIELS: Yes. We published that in the Journal of Infectious Diseases last year. Roger Shapiro summarized what I just did for vulnificus.

DR. MARGUERITE NEILL: What's the proportion? I mean, is it -- we've sort of been seeing it was roughly three or four to one. It's hard to come up with a hard number. But, roughly the sporadic case series looked like when they were reporting the other vibrio species, it was probably about three to one parahaemolyticus to others, and the majority of those were vulnificus. But, I'm talking about just for Texas for the same time.

DR. NICHOLAS DANIELS: For the same time frame. He reported about 345. They were very close in the number of infections, yeah. He went through ninety-six and he had around 320 something cases. From the Gulf Coast, just looking at Gulf Coast states, yeah.

DR. MICHAEL JAHNCKE: Other questions? Yes, Dane.

MR. DANE BERNARD: This is actually a question that you may not be able to answer until we get into some -- into the open session with some people more familiar with shipping than certainly I am. But, when we began to encounter vibrio cholera 01 from South America and there was a connection possibly with ballast water, I was under the impression that there was at least a recommendation, and I was under the impression it was implemented that ballast water be dumped twice in open water before ships came to port in the U.S. Has that not been continued? Was it ever implemented? It seemed to me to be a solution to that situation.

DR. NICHOLAS DANIELS: The International Maritime Organization from the U.N. did issue a statement that recommended that ships double-exchange ballast at sea, on high seas and not sort of in estuaries. Unfortunately that was -- it's voluntary and it's not enforced.

DR. MICHAEL JAHNCKE: Other questions? Morrie?

DR. MORRIS POTTER: Morris Potter. In your case series, Nick, from 1988 to 1997 you identified G.I. wound and septicemia, were those primary septicemias or did the

list of septicemias include those people who had primary G.I. disease that had secondary septicemias?

DR. NICHOLAS DANIELS: Those were all primary septicemia cases.

DR. MORRIS POTTER: In that same series two, or 17 percent of deaths had no reported underlying illness. Does CDC consider that to be lack of reporting of underlying illness, or that there is some marginal risk of death in those people who truly don't have underlying illness?

DR. NICHOLAS DANIELS: I think the question asked sort of known pre-existing illnesses. I mean, I think it's quite possible some of them might have had liver disease or had alcohol abuse and they could have been at risk, or had hepatitis. I think it could be just the reporting.

DR. MORRIS POTTER: One last question, and that is, it was stated frequently this morning that a majority of food isolates don't have virulence markers and are likely to be non-pathogenic. If this is true, if only a minority of food isolates are pathogens, is this going to eliminate outbreak data as a source of information on dose-response? Because one wouldn't then know how many of the vibrios consumer would have been pathogens.

DR. NICHOLAS DANIELS: That's a good question. Maybe the next presenter, who is going to talk about dose-response, could answer that.

DR. MICHAEL JAHNCKE: Any other questions from the subcommittee? If not, thank you, Dr. Daniels, for a very nice presentation. Thank you.

Our next speaker will be speaking about consumption. It's Dr. Michael DiNovi.

DR. MICHAEL DINOVI: Thank you. Good afternoon everybody. This portion of the risk assessment will consider the intake of raw molluscan shellfish. It should be short and fairly straightforward, because although this is a microbial risk assessment, the kinds of questions that I'm considering and the data inputs are the same as those for a typical chemical risk assessment or a safety assessment that we do in food additives all the time at FDA.

Well, since this is going to be a mathematical model, this module will receive an input distribution based on the data that you heard this morning. Probability of distribution function of vibrio levels in raw shellfish.

I will do some scientific magic here and my output distribution will be a probability distribution of

vibrio doses. Then we'll go on to Don's portion that you will hear next.

It's important to consider in any consumption scenario what is being eaten since this case was specifically restricted to raw molluscan shellfish. Although you've heard that vibrio can occur in all of these cases, we will solely be dealing with raw oysters and clams. This goes to answer a question that Bob Buchanan asked this morning about whether or not we going to consider the right half and the left half.

Until some information is passed to me to suggest that the vibrio in a shucked oyster is different from that that's consumed fresh shucked, there will be no difference.

To take a little further, we will really not be separating those two unless there's information that specifically allows us to.

Again, it's always important to consider when. You've heard this morning that these outbreaks occur mostly in the summers, and you can see I've just quickly reiterated that. There's nothing new here.

Again, unless something comes along to suggest that the vibrio contained in an oyster in December is somehow different from one in the summer, which is to say

it has a higher virulence factor, I'm using terms I don't understand, or it somehow is different, there will be no difference. There will be no time difference. Although it's clear that your risk from eating oysters is higher in the summer than it is in the winter, as far as consumption is concerned, the numbers that you eat will be the same.

The typical considerations that I make in any risk assessment are shown here. You always have to decide whether or not it's the total dose of whatever you're looking or how often you're dosed that matters. The question comes up whether or not it's an acute or chronic problem.

In this case it's pretty clearly an acute problem. I don't have any indication, I haven't heard anything to suggest that it's not. So what we will be considering are eating occasions, for the most part.

Based on some of the data that I've seen, and I have a lot of conflicting data, so you may see different numbers, that's one of the hopes I have from coming out of this meeting today is that I'll have better information when we actually get to doing the modeling.

The suggestion is that a typical single-meal contains 90 to 120 grams of oysters. Over a 3 or 14 day period, and I might point out that all of these data are

from publicly available databases, which is why they're limited. Over a three-day period the typical mean is 40 to 50 grams, and a 14 day intake is about 10 grams, which if you do the arithmetic shows you that most people will have one eating occasion of raw oysters or raw shellfish over a two-week period.

This goes to answer another question that was asked this morning. How many people are we talking about here? In 1993 FDA did a phone survey. I'd used numbers from the 1997 statistical abstract to come up with gross numbers. But, you can see approximately 50 million people reporting eating raw shellfish over the previous year of the phone survey. It's mostly men. More men than women. Demographically more Whites than Blacks. In the survey that we did "other" included Hispanic origin and Asian origin. That's why this number is so high at that time. But, approximately 50 million, and as you've seen this morning, in a number of cases those living near coastlines tend to eat more than those that live inland.

Although, this survey didn't differentiate people who were on vacation and may have consumed and then gone home. In the report that you saw in the previous talk that people eating in Galveston Bay reported from a wide variety of places, that would not have been caught in

this survey.

Five years after that survey we repeated it. This slide is meant to show a couple of things that have gone on. In the early nineties if you polled the public as to risk from food, pesticides and food additives would have come out on top. Microbial contaminates came out way on the bottom.

This decade has seen an enormous growth in education efforts both of federal and state regulators and others, and you see some of the outcomes of this year. All of these numbers have been reduced over the five years between the two surveys. Now it looks as though there's approximately, at least as of last year, were down to about 30 million people reporting eating.

This survey was meant to -- was designed to ask people if they knew of the risks of eating these foods, and as you heard previously, yes, people are. Perversely, the higher your level of education the less likely you are to be concerned about the risks that you are aware of. I don't know what this means exactly, but be that as it may, it won't factor into the risk assessment.

Since we're speaking acute intake, how often are people eating raw oysters? These are publicly-available data that I had access to. Again, I hope to get better

data in the next months that will be more to today's eating habits. Where you see a mean frequency this is from the MRCA, which is a marketing survey, of 1.4 eating occasions of all raw molluscan shellfish over a two-week period. Ninetieth percentile is approximately two. Five percent of people were reported as eaters over the two-week period. The number is obviously higher than that because you don't capture people. This is a fairly infrequently consumed food. Even though there are a lot of people eating it, eating occasions don't occur frequently. In fact, you see the maximum reported, two-week eating occasions was eight, and that is actually fairly low for most foods.

I have what I perceive as slightly better information from a 1994 Florida phone survey on eating habits. It suggested that the most common time for eating was one to two raw shellfish eating occasions over a six-month period, or once a month, twice a month, much less. And certainly more than once a week is very low.

These people were all eaters of raw molluscan shellfish so you even see that in any given year a third of those who eat are not reporting any eating occasions.

Reiterating earlier data, the eating occasion data that are publicly available from the U.S.D.A., showed

again about 110 grams. The Florida survey, which I'm leaning toward using more heavily because I think from the kind of data that you've seen this morning I think you'll agree it's not necessarily the case that the vibrio will be uniformly spread through any food. So, the actual number of oysters you eat is probably going to be more important. It looks like there will be some kind tri-mode of distribution. When you ask, how many did you eat, and you get specific answers, half-a-dozen, dozen, and two dozen are the numbers that show up most frequently in 60 percent of the cases. No surprise there.

The median in this survey was 13.8, a little higher than we heard this morning. And again going back to the 14 day average, which would suggest less than once, every period is only nine grams.

What factors involve raw oyster consumption? 90 percent of it is away from home. We heard that this morning.

From the FDA surveys we're aware that people are more aware -- people actually purchasing them are more of the risk than those that don't. The total is still low, at least it was in the survey. This, I believe, is the 1993, not the 1998 survey. Chicken and beef then were perceived as of higher risk.

People were asked about cooking as an alternative to eating raw shellfish. It was argued against on the basis of these two things: Raw shellfish is perceived as an appetizer. Cooked is perceived as a meal. I'm sure many of you have had the pitcher of beer and half dozen raw oysters, or whatever. That is desirable to the consumer. Many say that the taste is different. A raw oyster tastes different from a cooked oyster. I couldn't tell you, I have never eaten a raw oyster, but I will not let that prejudice me.

From the Florida survey in 1994, 40 percent reported consuming raw.

However, that survey already was showing changes. Three years prior in 1991 they had also completed the same survey. When people were asked -- let me see if I can say this so that it makes sense -- what percentage of people reported 100 percent of their eating occasions were raw, it went from 26 down to 23 percent. Where 50 percent were eating raw, 11 to 7, and when none were eating raw it was on the increase from 39 to 53 percent. You can see that educational efforts are indeed working.

As a validation of whatever distribution is derived at the end, it would be nice, in fact it would be

more than that, it'll practically be a requirement, that the integration of the curve of eating, if you imagine the number of eaters on one axis and the amount on the other, the total should be somewhat similar to the reported landing, some measure of the amount of oysters that have been consumed.

These data are taken from the National Marine Fisheries. They simply report three different types of oysters and where they're taken. You can see it's approximately 30 million pounds.

One bit of information that is missing from this that I do not have access or I did not have access to as of this morning is whether or not this is total weights or just meat weights. I'll need to clear that up beforehand. I've seen conflicting numbers that are much higher than this that I assume include shell weight.

That will essentially be the consumption module as it is. As I said, there will be an output distribution of dosage versus number of eaters, and that will be passed to dose-response module.

Thank you.

DR. MICHAEL JAHNCKE: Questions? I have one question for you. On your first couple of slides you went over your assumptions. Could you go over that again? I

sort of missed part of it.

DR. MICHAEL DINOVI: Chronic versus acute?

DR. MICHAEL JAHNCKE: Your assumption I think was your first two slides.

DR. MICHAEL DINOVI: What was eaten where?

DR. MICHAEL JAHNCKE: What was eaten and -- I'm trying to remember.

(Pause.)

DR. MICHAEL DINOVI: Normally when I do a consumption scenario these questions quickly fade into the background because you're -- for food additives, for example, there are no acute hazards, so everything is long term.

In this case there's a question in my mind as to how you eat oysters that matter. Someone mentioned this morning, if you eat the hot oyster at one sitting, does it matter if you eat another oyster within a given time frame? For the people reporting eating once a week, is there a different level of risk for those people than there is for someone who eats once every six months? I have no idea what the answer to that is. Presumably that information will come along and then I will taylor in my probability distribution function and take that into account.

So this is sort of a -- this is a question in my mind. It's not something I know the answer. I personally for myself answered the chronic question. I don't believe that you need to know what long-term how much meat weight you've eaten over your lifetime before you eat a bad oyster. That's what that specifically refers to. It appears as though it will be the individual oyster or small number of oysters that will -- I keep saying oysters, I mean raw shellfish -- that will matter. So that's what I was referring to here.

DR. MICHAEL JAHNCKE: Thank you. Other questions? Yes, Bob.

DR. ROBERT BUCHANAN: Bob Buchanan. I think I heard you mention early in your presentation that you're making the working assumption that the number of eating events is not seasonal, that it's evenly distributed throughout the year.

DR. MICHAEL DINOVI: No, no. I didn't suggest that. What I suggested was that an eating occasion in December will be no different from an eating occasion in July, unless I get information that suggests that that should be taken into consideration.

DR. ROBERT BUCHANAN: Do you have any data on the seasonality of consumption?

DR. MICHAEL DINOVI: At this moment, no, but it can be gotten.

DR. ROBERT BUCHANAN: Okay. What I might reinforce here is I would not assume that you're going to have some chronic effect associated with the consumption of oysters. I think you're going to be dealing almost exclusively with an acute single-event probability.

DR. MICHAEL DINOVI: Early on I asked within our group the question, does -- can you develop some kind of immunity if you're eating small amounts over a long period of time, and does that matter. The answer seemed to be no, or that there was no information to suggest that that was the case.

DR. MICHAEL JAHNCKE: Morrie?

DR. MORRIS POTTER: Perhaps a question that relates to that, that I might direct toward Peggy, we found during the early days of looking at campylobacter infections among raw milk consumers that those people who were chronic raw milk consumers were at lower risk because of G.I. immunity. Do we know anything about G.I. immunity for vibrios that would suggest that a chronic frequent consumer is at lower risk than a occasional consumer?

DR. MARGUERITE NEILL: Not that I know of. Not that I know from the states. I think it's one of the

things I've been kind of wondering about as I've been thinking about what the levels of other vibrios must be that people are exposed to both chronically and then acutely.

DR. MICHAEL JAHNCKE: Yes, Bob.

DR. ROBERT BUCHANAN: Morrie, maybe to answer not in a quantitative manner, but when we got comments on the previous speaker that he was saying why people ignored the risk was that I've been eating oysters all my life and I've never gotten sick, would tend to make you believe that if there is either a very low incidence of exposure, or there's very little probability of building up some kind of immunity.

DR. MICHAEL JAHNCKE: Other comments and questions? Yes, Dane.

MR. DANE BERNARD: Thank you. Review for me again why given the outbreak data which shows peaks during the warm months you would consider an eating exposure in December the same as you would consider one in August?

DR. MICHAEL DINOVI: It's much more likely that an eating occasion in December will have a much lower number of V.P. in the given amount of food, in the oyster that you eat. So eating one oyster in December you are very unlikely to find a large number of V.P., where one

oyster in July or October would be much higher. That's all I mean. I want to give the impression that there's nothing different about the V.P., the amount of V.P. in a given oyster at a given time. At least I'm unaware right now that there are differences. If there are differences that would have to be taken into account. That's simply a numbers difference.

MR. DANE BERNARD: How again are you going to account for -- or do we account for what was presented earlier in terms of the occurrence of, quote and unquote, virulent strains, two percent of total isolates seem to be TDH positive, which seems to correlate very well with isolates from infected persons? How do we account for that?

DR. MICHAEL DINOVI: As long as the numbers are proportional with the virulent strains to the non-virulent. Year around proportional. If it's always one or two percent you're again seeing a numbers difference, you're not getting enough of the virulent bacteria until the warm water raises all of the numbers. It floats the whole system.

It may well be that there are differences when the water temperature is -- maybe they grow differently, whatever, I'm not sure. But, at this point I don't have

any data to suggest anything else, I just assume it's the same.

One thing that I didn't mention which related to a question here. The susceptible population question. There's no way for me at this point to separate someone who is susceptible from someone who is not. I just don't have access to data on consumption of susceptible individuals.

Again, an unspoken assumption here is that everybody is the same. That is probably not the case. Someone with liver disease in July is probably at more risk than someone who is perfectly healthy at July from the same dosage. That will not be taken into account here.

DR. MICHAEL JAHNCKE: Bob?

DR. ROBERT BUCHANAN: I'm not sure I understand why it can't be taken into account. If you have a reasonable estimate of the proportion of the population at any one given time that is at increased risk, two percent of the population, and you can determine that their probability of getting, for example, septicemia is ten times more likely, I'm not sure I understand --

DR. MICHAEL DINOVI: (interrupting) Well, I would actually -- from the way I'm looking at where I'm

looking at an output distribution, I would say that would be a separate risk assessment. You would take that population and it would have a separate curve of likelihood of illness from a given dose.

I'm thinking in two dimensions here; given dose, probability of illness. If you are susceptible you would be on a completely different curve than someone who is not susceptible. I agree, given that you have a percentage and you can identify those people, you would simply say for these people this is the risk.

DR. ROBERT BUCHANAN: I guess the follow-up question is, is the plan of the risk assessment to look at a single biological endpoint? Are you going to be looking at multiple endpoints? The probability of gastroenteritis, the probability of septicemia, the probability of fatalities. Certainly if those three in a microbial risk assessment are key to then assessing not only incidence but also severity.

DR. MICHAEL DINOVI: Yeah. I can't speak for the whole risk assessment team, but I assume we will do something along those lines, yes.

DR. MICHAEL JAHNCKE: Yes, Morrie?

DR. MORRIS POTTER: To follow-up on what Bob was suggesting here, if for example we know the distribution

of consumers and it's 63 percent White male, we can also look at the health statistics for -- and weight the averages for liver disease, diabetes, and whatever risk factors we can identify, and try to reconstruct the population of consumers who would be at high risk of various endpoints.

DR. MICHAEL JAHNCKE: Bob?

DR. ROBERT BUCHANAN: Again, as a follow-up, I think it would be a very reasonable assumption to assume that with -- unless we had some additional data, that the eating habits of the at-risk population are the same as the non-at-risk population, I certainly think that we could make some kind of a breakdown on the risk associated with certain of these populations. I know it has been done before.

To follow-up your statement, that I think Dane was getting a little confused on, and to make sure that I understand you correctly, one of your earlier assumptions is that if I had an oyster with a million TDH positive vibrio in it and I consumed it in July, I would have the same risk if I consumed the same million in January.

DR. MICHAEL DINOVI: Exactly.

DR. ROBERT BUCHANAN: Okay.

DR. MICHAEL JAHNCKE: Any other questions?

Thank you, Dr. DiNovi. We'll take a break now for fifteen minutes. We'll return at 2:40.

(Whereupon, a recess was had in this matter.)

DR. MICHAEL JAHNCKE: If everybody would take their seats we will get started.

Our next speaker this afternoon is Dr. Donald Burr, and he will be speaking on dose response.

DR. DONALD BURR: Thank you very much. Hopefully, this will bring it to a close. It's been a long day, and again, we certainly appreciate the comments that have been coming in.

My task today is to look at the module within the public health risk characterization, that of dose response. In this section we're concerned with what information is available to support quantitative modeling of a dose-response relationship for parahaemolyticus. Hopefully, in the time that I'm up here we'll talk about some of the options that may be available for doing our modeling, and also, just point out a lot of the uncertainties. I think those have been coming up, at least in the round of questions. There are a lot of uncertainties and I hope that I bring up a lot more. I think that's the purpose of this, to try to get this input

to just see what we're missing and what we have out there.

In terms of dose-response relationship what are we trying to do? We're trying to relate levels of the biological agent ingested with frequency of infection or disease. So we're trying to get that critical link between exposure of the food and adverse human health outcomes.

As the second point points out, pretty much what Dr. Buchanan said, we really have to start looking at what is going to be the endpoint. Any model that's going to be developed has to first determine what that endpoint is going to be. It may be that you're just looking at infection, you're just looking at colonization without disease. It may be that you're looking for illness, just gastroenterology, or are you looking for a more severe disease. So any model is going to have to take that into account.

It's also important to point out that prediction of illness is a very multi-factorial, very difficult model to actually come out with something very certain. That's because it depends on three components. You've got a pathogen, you've got a host, and you've got an environment. All three of those things work individually, and all three of those interact with each other to effect

the infectious dose. That's going to come up at the end.

In fact, it may not be even possible to come up with a true infective dose. Because this would incorrectly imply that a single true infective dose for a population or a sub-population actually exists. You may have a minimal threshold dose, which unless reached will not cause human illness, but the actual dose which causes illness may vary according to those factors that I just mentioned.

In order to really look at disease we'd like to borrow a concept from the epidemiology literature and that's that of the disease triangle. As I just mentioned, in order to have disease, it depends on the interaction of these three components; the pathogen, the host, and the environment.

The pathogen may influence maybe the dose, as we know. Growth potential in the foods. Colonization potential. Pathogenicity of any particular strain. Serotypes, are there any differences? Increasing doses generally yield to increased risk, attack rates, and severity. But again, what is the difference between strains?

In terms of host, host influences on probability of illness include immune status, physiology, stomach, and

something we've heard a lot about already, pre-existing illness, pregnancy, nutritional status, age. Are there any gender effects that have to be looked at?

In terms of the environment. Environmental influences may include the food vehicle, consumption as a meal or just as a snack, the indigenous microbial floor within your intestinal tract, or the indigenous microbial competitors within the food. All these are going to get -- have to be taken into account when anyone is going to try and put a model together.

So in terms of starting out and developing a model what sources are out there that we have available that can be used in order to start these modeling processes?

On this slide it lists four different possibilities. We've heard a lot about the epidemiological outbreak investigations, and these are sort of our natural experiments, where we have outbreaks of food poisoning in people that are supposedly accidentally exposed in high enough numbers of people so that public health authorities go out and investigate.

Another source that may be used is a recent one described by actually Dr. Buchanan is using epidemiological and food survey data. We'll talk a little

bit about that.

The third option is to use feeding trials, human feeding trials. These are controlled experiments in which healthy volunteers are fed carefully quantitative doses of pathogens and their responses to that exposure are carefully monitored.

Finally, we have the use of surrogate models. These may be in humans, or they may be in animals. Either these other human feeding studies or other animal studies may provide a basis for extrapolating dose-response estimates back for vibrio parahaemolyticus.

We've heard a lot about epidemiological outbreak investigations. So what use are they? Most are not conducted with a degree of clinical or food microbiological evaluations. It's necessary that a single outbreak is going to be able to calculate a dose-response relationship. I think this is something Dr. Potter was sort of alluding to. How much real information can we get from them?

Recent outbreaks, as Nick just described, may indicate that the infectious dose may be less than ten-to-the-fifth CFU as opposed to what was historically thought.

In addition, there's epidemiologically -- well, there's been accidental laboratory infections, which also

may indicate an infectious dose of ten-to-the fifth CFU or less.

So there may be some outbreak data that may be available, maybe perhaps it can be of use to us.

This is again epidemiological and food survey data. This uses -- dose-response relationship is estimated on the basis of combining available epidemiological data with food survey data for ready-to-eat product.

So if you have any questions, just take them to Dr. Buchanan at the end.

What this does is actually takes the annual incidence of disease, levels of the pathogen in a ready-to-eat food, combines that with a consumption data in order to produce a conservative estimate of a dose-response relationship.

What they took as an example was out of Listeriosis. So they looked at the annual incidence of Listeriosis in Germany, they combined that with data on the levels of Listeria monocytogenes in smoked fish, which was ready-to-eat food. Then combined that with acid levels that are found on smoked fish and they generated a dose-response curve for this pathogen.

If we were to use this model we would take it as

some of the data that Andy presented, some of the data that Mike was talking about, that we would take into account levels of vibrio parahaemolyticus on raw oysters, data on the consumption of raw oysters, and data on disease incidents to generate a dose-response relationship.

Those are two options. The third option is human clinical feeding trials. As I said before, these are controlled experiments in which healthy volunteers are fed carefully quantitative doses of pathogens, and their response to the exposure is carefully monitored.

These days it's not just graduate students. There's more people that get involved in these at the present time.

Several feeding studies have been performed with vibrio parahaemolyticus. I'd like to, in the next group of slides, describe five of these studies.

The first study occurred in 1958, and this is by Takikawa. In this case he tested a single Kanagawa positive hemolytic strain. The doses were ten-to-the-six, ten-to-the-seventh, and you see in terms of response, at ten-to-the-six we got one out of two, and in ten-to-the-seven, two out of two came out with diarrhea.

In this particular study not much is known on